

A<sup>1</sup> (contd.)  
identical in the three *csp* mRNAs are shown in bold letters. The 13-base homologous sequence is *cspA*, *cspB*, *cspG*, and *cspI* are boxed (the upstream box). Positions of the SD sequence and the initiation codon are underlined. Potential base pairing between *cspA* mRNA and 16S rRNA are indicated by vertical lines. Positions of RNase V1 sensitive sites (Powers *et al.*, 1988) are dotted.

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**Please replace the last paragraph on Page 9 with the following:**

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A<sup>2</sup>  
Fig. 14 shows a comparison of the secondary structures of the 5'-UTRs for the deletion constructs. Secondary structures of the 5'UTR for each deletion construct were predicted with a nucleotide sequence analysis program (DNASIS-Mac; Hitachi Software Engineering Co. Ltd.) based on the method of Zuker and Stieger, 1982. Nucleotides are numbered as the position in the *cspA* mRNA starting from the transcription initiation site as +1. The position of the deletion in each mutant is shown by an arrow with the nucleotide numbers of the deleted region. The highly conserved 13-base sequence upstream of the SD sequence designated the upstream box are boxed. The initiation codon and the SD sequence are also boxed. The *cspA* 5'UTR deletion constructs: (A) pMM67 (SEQ. ID. NO. 55); (B) pMM022 (SEQ. ID. NO. 56); (C) pMM023 (SEQ. ID. NO. 57); (D) pMM024 (SEQ. ID. NO. 58); (E) pMM025 (SEQ. ID. NO. 59); and (F) pMM026 (SEQ. ID. NO. 60).

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**Please replace the paragraph corresponding to the first paragraph on page 10 with the following:**

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Fig. 15 shows enhancement of *cspB* translation by DB. (A) *cspB*-DB-anti-DB complementarity: the *cspB*-DB sequence (SEQ. ID. NO. 61) is boxed and encompasses the region from codons 5 to 9 (Mitta *et al.*, 1997). Nucleotides 1481-1443 of 16S rRNA (SEQ. ID. NO. 62) are shown. Additional *cspB* mRNA-16S rRNA possible base pairings

downstream of DB are also shown. The AUG codon is circled, the SD sequence is boxed and L-shaped arrows show the positions where the *cspB* gene was fused to *lacZ*. (B) Translational *cspB-lacZ* fusion constructs. On the top, the *E. coli cspB* gene is depicted from its 5' end. In pB3, pB13 and pB17, the *lacZ* gene is fused to *cspB* at residue +177 (3 aa), +200 (13 aa) and +212 (17aa), respectively. The pB13sd and pB17sd are the same as pB13 and pB17, respectively, except that their SD sequences are changed from 5'-AGGA-3' to 5'-CTTC-3'. (C)  $\beta$ -galactosidase activity of the *cspB-lacZ* constructs obtained before (time 0) and after (1, 2 and 3 hr) temperature shift from 37 to 15°C. *E. coli* AR137 cells were transformed with pB3, pB13, pB13sd, pB17 and pB17sd were grown in medium, and at mid-log phase ( $OD_{600} = 0.4$ ) cultures were shifted from 37 to 15°C.  $\beta$ -galactosidase activity was measured. (D) mRNA levels of pB3, pB13, pB17 or pB13sd after temperature shift from 37 to 15°C: the *cspB-lacZ* mRNAs were detected by primer extension before temperature downshift (time 0) and at 1, 2 and 3 hrs after temperature shift. (E) mRNA stability from pB3, pB13, pB17 and pB13sd: *E. coli* AR137 cells transformed with pB3, pB13, pB17 and pB13sd were grown under the same conditions described above. At mid log phase, the culture was shifted to 15°C and after 30 min., rifampicin was added to a final concentration of 0.2 mg/ml (time 0). Total RNA was extracted at 5, 10 and 20 min. after rifampicin addition. The *cspB-lacZ* mRNAs were detected by primer extension.

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**Please replace the paragraph bridging pages 10 to 11 with the following:**

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**Fig. 16** shows the effect of a perfectly matching DB enhancing the translation of *cspA*. (A) Translational *cspA-lacZ* fusion constructs. The *cspA* gene structure from its 5'-end is shown at the top. pJG78DB1 and pJG78DB2 were constructed from pJG78 as described in Experimental Procedures. The DB sequences of pJG78DB1 (SEQ. ID. NO. 64) (12 matches) and pJG78DB2 (SEQ. ID. NO. 65) (15 matches) are shown at the bottom with the

16S rRNA anti-DB sequence (SEQ. ID. NO. 63). **(B)**  $\beta$ -Galactosidase activity of the *cspA-lacZ* fusion constructs after cold shock at 15°C. *E. coli* AR137 cells transformed with pJIG78, pJIG78DB1 or pJIG78DB2 were grown in LB medium, and at mid-log phase ( $OD_{600}=0.4$ ) cultures were shifted from 37°C to 15°C.  $\beta$ -galactosidase activity was measured before (time 0) and 1, 2 and 3 hr after the shift. **(C)** Detection of the *cspA-lacZ* mRNAs. Total RNA from *E. coli* AR137 cells carrying pJIG78, pJIG78DB1 or pJIG78DB2 was extracted at the same time points indicated above and used as a template for primer extension. **(D)** mRNA stability from the *cspA-lacZ* constructs. *E. coli* AR137 cells transformed with pJIG78, pJIG78DB1 and pJIG78DB2 were grown as described above. At mid-log phase, the cultures were shifted to 15°C and after 30 minutes rifampicin was added to a final concentration of 0.2 mg/ml (time 0). Total RNA was extracted at 5, 10 and 40 minutes after rifampicin addition. The *cspA-lacZ* mRNAs were detected by primer extension.

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Please replace the paragraph bridging pages 11 to 12 with the following:

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**Fig. 17** shows that a perfectly matching DB enhances translation at 37°C: **(A)** pIN-*lacZ* constructs. The *XbaI-SalI* fragment from pJIG78 or pJIG78DB2 was inserted into the *XbaI-SalI* sites of pIN-III to create pINZ and pINZDB1, respectively which then were used to create pINZDB2, pINZDB3 and pINZDB4. **(B)** mRNA sequences of the pIN-*lacZ* constructs showing the position of SD, AUG and DB. The *lacZ* in pJIG78 has a 10-match DB. The perfect match DB located after the 5<sup>th</sup> codon has 16 residues complementary with the anti-DB. The pin-*lacZ* constructs: pINZDB1 (SEQ. ID. NO. 68); pINZDB2 (SEQ. ID. NO. 69); pINZDB3 (SEQ. ID. NO. 70); and pINZDB4 (SEQ. ID. NO. 71). 16S rRNA anti-DB (SEQ. ID. NO. 67). pJIG78 (SEQ. ID. NO. 66). **(C)**  $\beta$ -Galactosidase activity of the pINZ-*lacZ* constructs. Cultures of *E. coli* AR137 cells transformed with pINZ, pINZDB1,

*AS (cont'd)*  
pINZDB2, pINZDB3 and pINZDB4 were grown at 37°C under the same conditions described in Figure 1. IPTG (1 mM) was added at mid-log phase to each culture.  $\beta$ -Galactosidase activity was measured before (time 0) and at 0.5, 1, 2 and 3 hr after IPTG addition. **(D)** Rate of  $\beta$ -galactosidase synthesis of the pINZ-*lacZ* constructs. Cultures of *E. coli* AR137 cells carrying pINZ or pINZDB1 were grown at 37°C under the same conditions described above. IPTG (1mM) was added at mid-log phase to each culture. Rate of  $\beta$ -galactosidase synthesis was measured before (time 0) and 0.5, 1, 2, 3 and 4 hr after IPTG addition. Cells were pulse-labeled with trans-[<sup>35</sup>S]-methionine. Cell extracts from each time point were analyzed by 5% SDS-PAGE and the  $\beta$ -galactosidase synthesis was measured by phosphorimager. The ratio of  $\beta$ -galactosidase synthesis of pINZ and pINZDB1 is shown at each time point.

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